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Introduction: The clinical impact of anti-HLA antibodies is one of the major area of research in renal allograft transplantation. Luminex Platform has emerged as the favoured technology for detection of HLA antibodies in addition to the CDC crossmatch (XM). Luminex DSA crossmatch has been recently introduced in India for detecting antibodies that are missed by CDC XM. We present our experience with the Luminex based DSA crossmatch test done in the recipients who underwent transplantation at Sir Ganga Ram Hospital in the year 2013-14 with one year post transplant follow up. The objective of the study was to evaluate the impact of the pre-Tx DSAs detected by Luminex crossmatch on the clinical outcome of the renal graft over a period of one year

Methods: We began performing DSA (donor specific antibody) monitoring protocol by Luminex in the year 2013 at our center. The present study includes patients from January 2013 to December 2013 with one year follow up post kidney transplant. Pre- transplant sera from 46 renal transplant recipients with a negative CDC crossmatch were assessed for donor-specific antibodies (DSA) detection on Luminex Platform using Lifecodes DSA kit (Immucor). The serum samples with DSA (HLA-Class I or II or both) of MFI (mean fluorescent intensity) value more than 500 was considered to be positive. The results were then correlated with the clinical outcomes of the renal allograft.

Results: SAs were found in 11 out of 46 recipients (23.9%). Of the eleven DSA positive patients, 3 patients (27.27%) developed acute graft rejection. All these 3 patients had positive C4d staining in their biopsies and the MFI value of the DSA on Luminex platform was found to be more than 1000. The remaining 8 DSA positive patients showed no rejection and had stable graft function. The MFI value of the DSAs in these patients ranged from 500-1000. All the 35 DSA negative patients (76.1%) were also having stable graft function in one year follow up. Hence, AMR was more frequent in the DSA positive group than in DSA negative group. outcome of the renal graft over a period of one year.

Conclusions: The present study evaluates the importance of Luminex DSA crossmatch test in detecting the donor specific HLA antibodies over the CDC crossmatch. There was a higher incidence of AMR in patients with pre-transplant DSA despite a negative CDC crossmatch. The present study clearly establishes that the Luminex DSA crossmatch is helpful for predicting post transplant graft outcome or rejection. The laboratory cut off value of the MFI for positive DSA was increased from 500 to 1000. We suggest that DSA MFI value above 1000 should be considered for further evaluation by Single antigen bead assay (SAB) by Luminex. However, the clinical impact of the pre-Tx DSAs detected by Luminex techniques has to be fully evaluated in terms of graft survival and more retrospective studies with larger sample size.

P14

MIF IS AN ENDOGENOUS FIBROSIS LIMITING FACTOR IN PROGRESSIVE KIDNEY DISEASES

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Introduction: Renal fibrosis is the underlying process and the common end-point of progressive kidney diseases. A multitude of factors and processes were found to promote and aggravate renal fibrosis, but our understanding of endogenous factors limiting fibrosis is limited.

Methods: Here we analyzed the role of macrophage migration inhibitory factor (MIF), a pleiotropic proinflammatory cytokine, in animal models of renal tubulointerstitial fibrosis and inflammation.

Results: We show that MIF expression is reduced in murine and human renal tubulointerstitial fibrosis. MIF inhibition using gene knock-out, neutralizing antibodies or a small-molecule inhibitor aggravated fibrosis and worsened parameters of renal function, while treatment with murine recombinant MIF abrogated renal fibrosis. This effect was consistent in three distinct models of renal fibrosis, i.e. those induced by obstruction, ischemia-reperfusion or toxin, and was also effective when treatment was initiated in already established fibrosis. Bone-marrow chimeras showed that local but not bone-marrow derived MIF was involved. Mechanistically, MIF reduced the expression of chemokine MCP-1 in tubular cells and reduced renal inflammatory infiltrates already early in disease.

Conclusions: Taken together, we identified a hitherto unappreciated role of MIF as an endogenous factor limiting renal tubulointerstitial fibrosis via limiting renal inflammation. Our data raise important safety concerns regarding the envisaged use of MIF inhibition as a treatment for inflammatory diseases.

P15

THE EFFECT OF PEPTIGYLARGININE DEIMINASE 4 INHIBITOR ON MPO-ANCA PRODUCTION IN MOUSE MODEL

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Introduction: MPO-ANCA-associated vasculitis (MPO-AAV) is a systemic small vessel vasuculitis that involves preferentially the kidneys. It has been shown the pathogenic role of MPO-ANCA in MPO-AAV. Neutrophil extracellular traps (NETs), which are released from neutrophils activated by microorganisms, are composed of net-like chromatin fibers and antimicrobial proteins, such as MPO. The activated neutrophils die in due course with the formation of NETs. During the NET formation, histones bound chromatin fibers are citrullinated by peptigylarginine deiminase 4 (PAD4), and consequently the chromatin fibers are decondensated. Thus, PAD4 plays a pivotal role in the NET formation. Although NETs are essential for elimination of microorganisms, excessive formation of NETs has been shown to be implicated in the MPO-ANCA production. The aim of this study is to determine that inhibition of PAD4 can suppress the NET formation and MPO-ANCA production in vivo.

Methods: According to our previous report, NETs were induced in peripheral blood neutrophils derived from healthy donors (1×106/ml) by stimulation with 20 nM phorbol myristate acetate (PMA) with or without 20 μM anti-thyroid drug, propylthiouracil (PTU) for 2 hours at 37 °C. The effect of PAD4 inhibitor, 200 μM Cl-amidine, on the in vitro NET formation induced by 20 nM PMA

with or without 20 μM PTU was determined. Next, we established mouse models with MPO-ANCA production. BALB/c mice were given intraperitoneal (i.p.) injection of PMA (50 ng at day 0 and day 7) and oral administration of PTU (5 mg/day) for 2 weeks. In this model, MPO-ANCA was produced by day 14 though an obvious vasculitic phenotype was not observed. These mice were divided into two groups, namely Group 1 with daily i.p. injection of Cl-amidine (0.3 mg/200 μl/day) (n=7) and Group 2 with daily i.p. injection of PBS (200 μl/day) (n=13). Two weeks later, serum MPO-ANCA titers and amounts of peritoneal NETs were compared between the 2 groups.

Results: In vitro NET formation induced by 20 nM PMA with or without 20 μM PTU was inhibited significantly by 200 μM Cl-amidine. Serum MPO-ANCA titers of Group 1 mice (32.3 ± 31.0 ng/ml) were significantly lower than those of Group 2 mice (132.1 ± 41.6 ng/ml). The amounts of peritoneal NETs in Group 1 mice was significantly smaller than those in Group 2 mice. These findings suggested that the NET formation was inhibited significantly by Cl-amidine both in vitro and in vivo, and that the MPO- ANCA production was also suppressed by Cl-amidine in vivo.

Conclusions: PAD4 inhibitor suppresses MPO-ANCA production through inhibition of NET formation in mouse model so that it could be a novel therapeutic modality for MPO-AAV in humans.

P16

INTERCELLULAR ADHESION MOLECULE-1 K469E(A/G) POLYMORPHISM AND ITS EFFECTS IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

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Introduction: Recent research has implicated that inflammation may be a key pathophysiological mechanism in diabetic nephropathy (DN), although its pathogenesis is multifactorial. Intercellular adhesion molecule 1 (ICAM-1) is an acute phase marker of inflammation and the ICAM-1 gene is located on chromosome 19p13.2 and resides a linkage region to diabetes and DN. To investigate whether ICAM-1 has effects in the development of DN, we have recently performed genetic and pathological studies of this molecule in Swedish and Malaysian subjects with normal glucose tolerance (NGT), diabetes and DN.

Methods: We genotyped six single nucleotide polymorphisms (SNPs) in the ICAM-1 gene with TaqMan allelic discrimination. We also determined plasma ICAM-1 levels with an enzyme-linked immune-sorbent assay kit.

Results: We found that non-synonymous SNP rs5498 (K469E A/G) was associated diabetes and DN and the G allele had a protective effect. Particularly, we found a high heterozygous index of this polymorphism presenting in both populations. The genotype distribution of this polymorphism was kept in Hardy-Weinberg Equilibrium and no duplicon in the genomic sequence was found. The ICAM-1 K469E(A/G) polymorphism resides in the 5th Ig-like domain of ICAM-1 protein. This domain is essential for dimerization, surface presentation and solubilisation of proteins of the protein and subsequently plays a crucial role in the activity of ICAM-1 protein in the interaction with LFA-1 and the adhesion of B cells. Furthermore, we found the carriers with heterozygous genotype had higher fasting glucose levels among newly diagnosed type 2 diabetes patients compared with the subjects with wild or mutant homozygous genotype. Plasma ICAM-1 levels were increased from the subjects with NGT, diabetes without DN to the patients with DN. Among diabetic patients with DN, the carriers with heterozygous genotype had higher plasma ICAM-1 levels compared with other patients.

Conclusions: Our study provided evidence that ICAM-1 has effects in the development of DN. The patients carrying with heterozygous genotype of SNP rs5498 (K469E A/G) in the ICAM-1 gene have higher risk susceptibility to DN. The combined approach with genotyping this polymorphism and measuring plasma ICAM-1 levels may be useful for prediction of DN in translation medicine.

P17

INTRAVASCULAR NEUTROPHIL EXTRACELLULAR TRAP (NET) RELEASE PROMOTE VASCULAR INJURY AND TUBULAR NECROSIS UPON ISCHEMIA/REPERFUSION INJURY (IRI) OF KIDNEY

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Introduction: Acute tubular necrosis (ATN) is common in severe acute kidney injury (AKI). Infiltrating neutrophils contribute to the crescendo of renal inflammation and kidney injury (necroinflammation), but how neutrophils contribute to ATN is not clear. We hypothesized that infiltrating neutrophils release neutrophil extracellular traps (NETs), which implies the release of cytotoxic DAMPs like histones that accelerate ATN and AKI.

Methods: In vivo; Postischemic AKI was induced in wild type mice by unilateral clamping of the renal pedicle for 35 minutes followed by reperfusion for 6h or 72h. Formation of NETs was identified by immunostaining using citrullinated histone 3 (CitH3) antibody. Intravascular NETs were confirmed by neutrophil elastase (NE)-DNA complex ELISA in plasma. Renalcell death was evaluated in kidney sections by Tunel staining and PicoGreen DNA assay of plasma. To investigate the effect of NETs inhibition in bilateral IRI (ischemic 35min, reperfusion 24h) group of mice were treated with either PAD inhibitor (PADin) or neutrophil depletion and sacrificed 24h after reperfusion. In vitro; To investigate whether hypoxia can directly induce NETs formation or indirectly via hypoxia-induced tubular cell death, 1) human neutrophils were incubated in 1% or 20%O2 for 24h, 2) the media of tubular cell line (TC), which were incubated in 1% or 20%O2 for 24h, and stimulated with neutrophils for 4h. Furthermore, PMA or histone- induced NETs were treated by PADin, anti-histone antibody and heparin. Histone-stimulated neutrophil media were applied to TC. NETs and TC injury in vitro were evaluated by CitH3 staining/MPO-DNA complex and LDH assay, respectively.

Results: IRI kidney showed increased positivity for CitH3 in areas of tubular necrosis of the outer medulla. Plasma levels of the NE-DNA complex were increased in a time-dependent manner as compared to sham-operated mice. NET-induced renal cell death was shown in terms of increased Tunel positive area in kidney and plasma DNA 6h after reperfusion compared to the sham group. Subsequently, NETs in plasma and kidney increased 15～24h after reperfusion. In bilateral IRI, treatment with both PADin and neutrophil depletion significantly reduced the plasma levels of NE-DNA complex and NETs area in kidney compared to the vehicle group. Treatment further improved renal excretory function in terms of reducing plasma creatinine levels. In vitro, the NET-